International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 364-368, Jan-Mar 2012

Phytochemical and HPTLC Studies of Various Extracts of *Annona squamosa* (Annonaceae).

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Abstract: The leaves of the plant *Annona squmosa* were collected, powdered and extracted successively with different solvents. The extracts were subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins, and steroids. The TLC and HPTLC techniques were used for qualitative determination of possible number of components in the various extracts. Solvent systems for all the extracts were optimized in order to get maximum separation on plate. Presence of various phytochemicals was confirmed by the use of different spraying reagents.
Keywords: *Annona squmosa,* TLC, Phytoconstituents, HPTLC.

Introduction

Annona squamosa Linn. (Annonaceae) commonly known as sitaphal in Hindi and custard apple in English, is a small tropical tree that is native to South America. It is a shrub or tree, having height upto 8 m, trunks short, not buttressed at base. Traditionally, bark decoction is used to stop diarrhoea, while the root is used in the treatment of dysentry. A decoction of the leaves is used as a cold remedy and to clarify urine. The fruits of *Annona* are haematinic, cooling, sedative, stimulant, expectorant, maturant, and tonic. They are useful in anaemia, burning sensation. The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair[1,2]. Scientific investigations have shown that the crude extract possesses miticidal[3], antifeedant[4], antidiabetic[5] and anxiolytic[6] activities.

Experimental

Materials and Method

The plant *Annona squamosa* Linn. were collected from the field of Punjabrao Krishi Vidyapith, Nagpur, India, in the 1st week of August. It was authenticated from Department of Botany, Nagpur University, Nagpur. Its herbarium is deposited in the above department (Voucher specimen no.9089).

Preparation of Extracts

The leaves of *Annona squamosa* were dried in shade under normal environmental condition and homogenized to coarse powder and stored in opaque screw tight jars until use. Powdered drug was charged into soxhlet apparatus and extraction was carried out with following solvents succesively.

1) Petroleum ether (40-60°C), 2) Chloroform, 3) Ethyl acetate, 4) Acetone, 5) Methanol

Each time before employing the solvent of higher polarity marc was dried. Each extract was then concentrated using rotary vaccum evaporator at 40-50°C under vacuum and dried residue was collected in an opaque glass bottles for further studies. Percentage practical yield of petroleum ether (40-60°C), chloroform, ethyl acetate, acetone, and methanolic extracts were found to be 3.85, 2.33, 2.39, 1.2, 7.07 % w/w respectively.

Preliminary Phytochemical Screening
The plant may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins and flavonoids. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, all the extracts were subjected to battery of chemical tests.[7]

Thin Layer Chromatography
All the extracts of *Annona squamosa* were subjected to thin layer chromatographic studies, to determine the probable number of compounds present.

Preparation of the plates:
The precoated TLC plates (Merk, Germany) made up of silica gel G as an adsorbent ,was activated in an oven for 30 minutes at 110°C. Test samples (1mg/ml of all extracts in respective solvents) were applied in the form of bands using Linomat IV applicator.

Development of solvent system
A number of solvent systems were tried [8, 9], in order to get maximum separation on plate. After development of plates, they were air-dried and numbers of spots were noted & R_f values were calculated. Spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Ferric chloride reagent, for flavonoids, Dragendorff"s reagent for alkaloids, Liebermann-Burchard reagent for steroids and Anisaldehyde- sulphuric acid for sugars.

High Performance Thin Layer Chromatography
Precoated plates were used for application of sample. Known quantities of each extracts were dissolved in respective solvent and samples were applied in precoated plate with the help of Linomat IV applicator. Solvent systems optimized for TLC study was chosen for HPTLC study.

Chromatographic conditions:
Following are the chromatographic conditions required to get an effective resolutions by selected mobile phase.
Stationary phase: HPTLC precoated, silica gel G 60 F254 (Merck, Germany)
Size: 10 x 10 cm
Developing chamber: Twin trough glass chamber
Mode of application: Band
Band size: 5 mm
Separation technique: Ascending
Temperature: 20 ± 5O°C
Saturation time: 30 min
Scanning wavelength: 254 nm / 366 nm
Scanning mode: Absorbance/Reflectance.

Table 1: Preliminary phytochemical screening

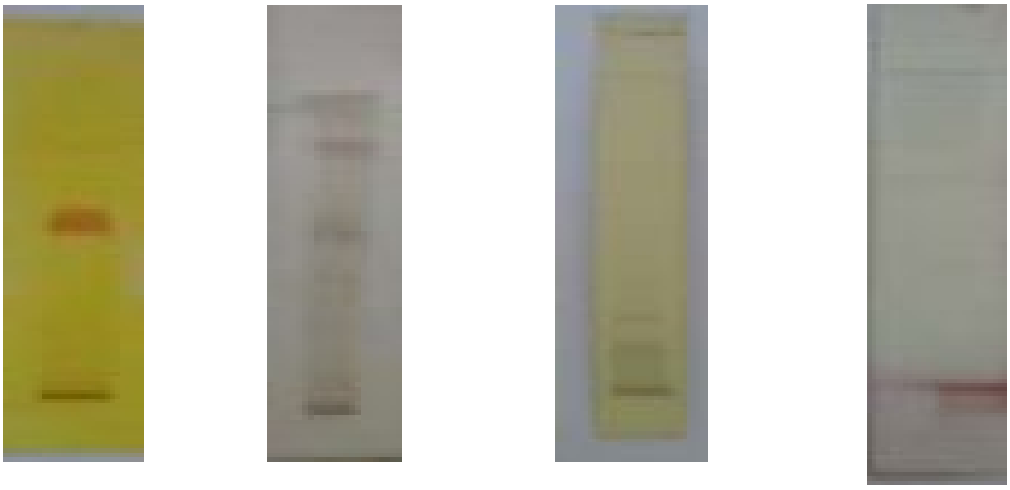
Sr. No.	Plant constituent	Test/Reagent	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Acetone extract	Methanolic extract
01	Steroids	Salkowski reaction Liebermann-Burchard test	+ +	+ +	+ +	– –	– –
02	Alkaloids	Dragendorff"s reagent Mayer's reagent Hager's reagent Wagner's reagent	– – – –	– – – –	– – – –	– – – –	+ + + +
03	Tannins	Ferric chloride test Lead acetate test Potassium dichromate	– – –	– – –	– – –	– – –	+ - +
04	Flavonoids	Shinoda test	–	–	+	+	+
05	Carbohydrates	Molish's test Barfoed's test	– –	– –	+ –	+ –	+ –
06	Proteins	Biuret test Xanthoproteic test	– –	– –	– –	– –	+ –
07	Saponins	Foam test	–	–	–	+	+

+ :- Found to be present
– :- Found to be absent

Table 2: Thin Layer Chromatography

Test extract	Solvent system	Number of Spots	R_f values
P.E	Hexane: Ethyl acetate (8:2)	06	0.18,0.30,0.41, 0.45,0.59,0.83
Chloroform	Chloroform: Methanol (9:1)	03	0.58,0.75,0.83
E.A	Chloroform: Methanol: Formic acid (8:1:1)	04	0.02,0.10,0.18,0.28
Acetone extract	Chloroform: Methanol: Formic acid (7:2:1)	05	0.35,0.45,0.53, 0.61,0.78
Methanol	Chloroform: Methanol (8:2)	04	0.05,0.15,0.22, 0.34

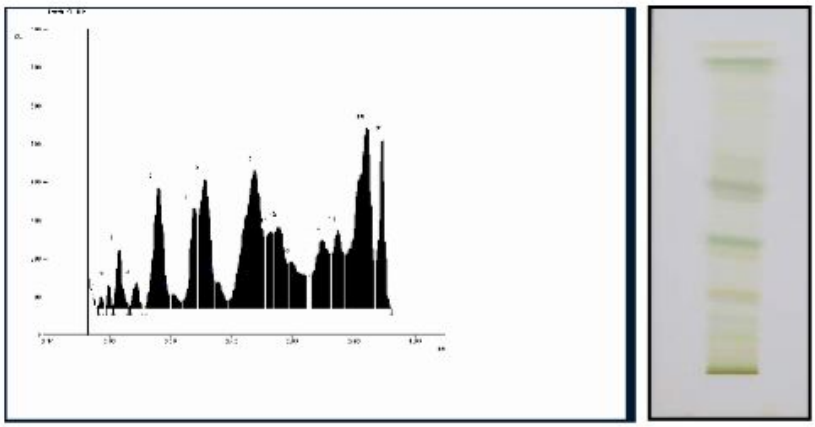
Figure 1



Dragendorff's Liebermann-Burchard 5%Ferric chloride Anisaldehyde-sulphuric acid

Orange color Red color Green color Red color

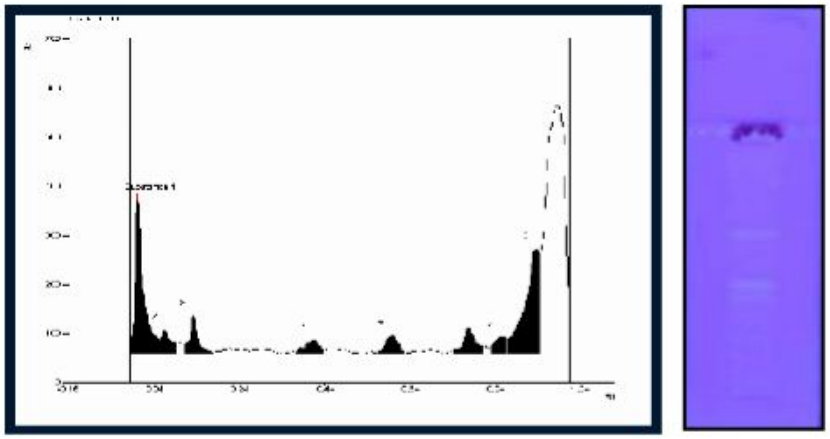
Figure 2



HPTLC of pet ether extract of *Annona squamosa*

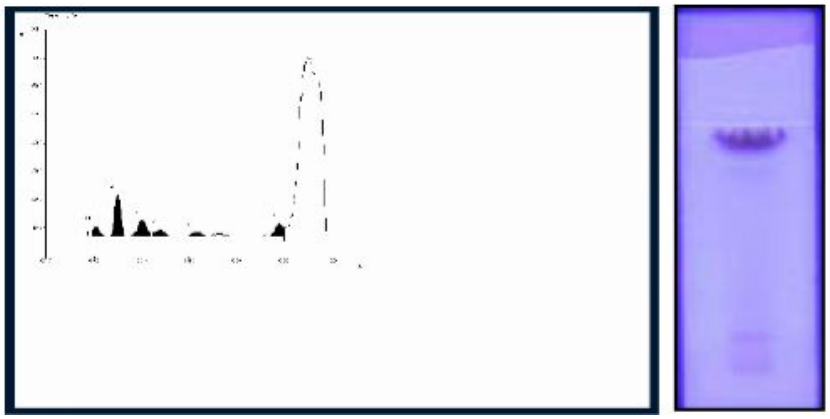
17 compounds were separated having R_f values 0.02, 0.04, 0.07, 0.12, 0.18, 0.26, 0.30, 0.35, 0.41, 0.45, 0.57, 0.59, 0.65, 0.72, 0.78, 0.83, 0.93

Figure 3



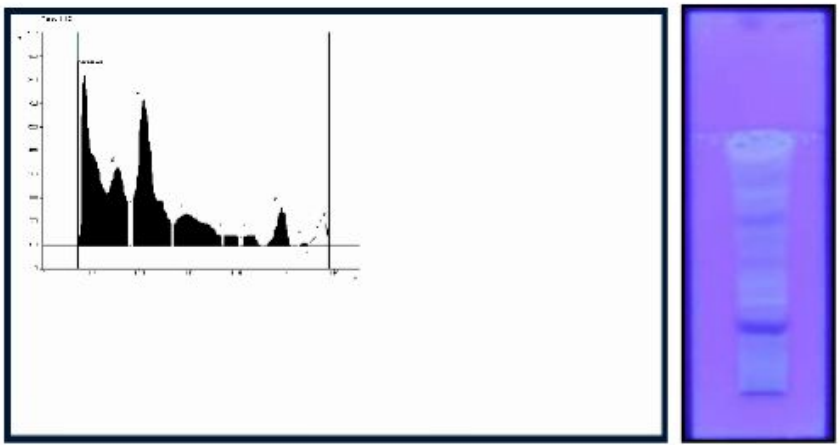
HPTLC of chloroform extract of *Annona squamosa*
5 compounds were separated having R_f values 0.58, 0.75, 0.83, 0.90, 0.96

Figure 4



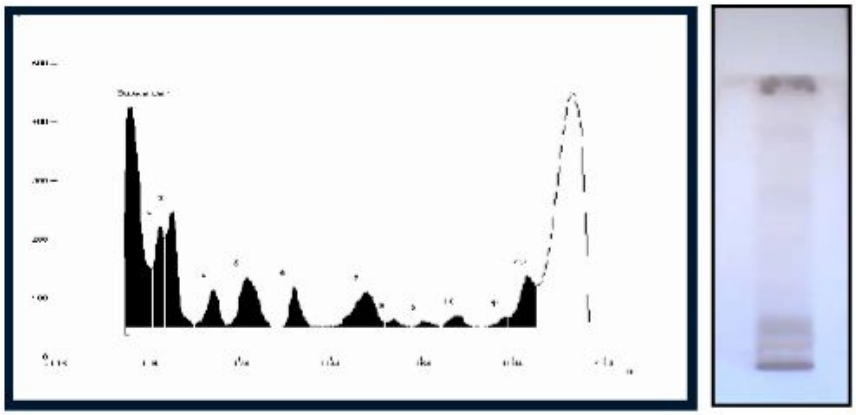
HPTLC of ethyl acetate extract of *Annona squamosa*
6 compounds were separated having R_f values 0.02, 0.10, 0.18, 0.28, 0.43, 0.77

Figure 5



HPTLC of acetone extract of *Annona squamosa*
9 compounds were separated having R_f values 0.10, 0.20, 0.29, 0.35, 0.38, 0.45, 0.53, 0.61, 0.78

Figure 6



HPTLC of methanolic extract of *Annona squamosa*
11 compounds were separated having R_f values 0.05, 0.08, 0.15, 0.22, 0.34, 0.45, 0.56, 0.62, 0.69, 0.79, 0.83

Result and Discussion

Preliminary phytochemical screening of various extracts revealed the presence of different primary and secondary metabolites. *Annona squamosa* leaves were found to contain steroids, flavonoids, carbohydrates, saponins, alkaloids and tannins (Table1).

All the extracts were subjected to TLC and HPTLC studies to estimate number and type of phytoconstituents present in it. Number of solvent systems were tried, however good resolution was obtained in the solvent system mentioned in table 2. In the optimized solvent system Pet ether extract showed chloroform extract -5, ethyl acetate extract -6, acetone extract -9 and methanolic extract showed -11 bands (Figure 2-6).

Presence of phytoconstituents in particular extract was confirmed by spraying TLC plates with different spraying reagents. Presence of steroid was detected visually by spraying with Liebermann-Burchard reagent (red color), flavonoids with ferric chloride solution (green cooler) where as carbohydrates were confirmed by spraying with Anisaldehyde- sulphuric acid (red color). Alkaloids show orange color on spraying with dragendorff"s reagent (Figure 1).

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